

# Optimization of Steam Pretreatment of SO<sub>2</sub>-Impregnated Corn Stover for Fuel Ethanol Production

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## Abstract

In this study, corn stover with a dry matter content of 20% was impregnated with SO<sub>2</sub> and then steam pretreated for various times at various temperatures. The pretreatment was evaluated by enzymatic hydrolysis of the solid material and analysis of the sugar content in the liquid. The maximum overall yield of glucose, 89% of the theoretical based on the glucan in the raw material, was achieved when the corn stover was pretreated at 200°C for 10 min. The maximum overall yield of xylose, 78%, was obtained with pretreatment at 190°C for 5 min.

**Index Entries:** Steam pretreatment; SO<sub>2</sub>; corn stover; fuel-ethanol production.

## Introduction

Ethanol produced from various biomasses is a renewable energy source. The net contribution of CO<sub>2</sub> to the atmosphere is, in theory, zero because the same amount of CO<sub>2</sub> is assimilated during the growth of biomass as is released during combustion. Our dependency on oil is also lowered, thus improving self-sufficiency. Ethanol can be used not as only fuel in engines adapted to ethanol use but it can also be added to petrol in concentrations up to 20% without harming a normal combustion engine. Mixing ethanol and petrol also lowers toxic emissions by boosting the octane number, thus replacing other toxic octane-enhancing compounds. Ethanol addition also provides more oxygen resulting in more complete combustion (1). However, the production cost of bioethanol must be closer to that of fossil fuels in order to become commercially viable. The raw material must be cheap and ethanol production must be optimized.

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Ethanol can be produced from lignocellulosic material by fermentation of the monosaccharides liberated from cellulose and hemicellulose. *Saccharomyces cerevisiae* has proved to be a yeast strain very well suited to the fermentation of lignocellulosic hydrolysates (2) but naturally occurring strains only ferment glucose and mannose. It may, however, utilize galactose after adaptation. Corn stover, which is the corn stalk and leaves left when the corn cobs are harvested, is an abundant agricultural by-product with low commercial value. Apart from glucose, corn stover also contains large amounts of xylose, and although several attempts have been made to genetically modify different microorganisms to ferment pentoses (3), the strains are still in the laboratory stage (4,5). Pentoses may contribute significantly to the overall yield of ethanol from corn stover, and it is thus important to find means of also utilizing also this fraction.

Lignocellulosic materials are very recalcitrant and not easily hydrolyzed by enzymes. One way of liberating the monosugars from the cellulose and hemicellulose structure is by using some kind of pretreatment followed by enzymatic hydrolysis. The function of pretreatment is thus to make the cellulose accessible to the enzymes and to hydrolyze the hemicellulose, ideally without further degradation. The most investigated pretreatment method is steam pretreatment, which can be carried out with or without the addition of an acid catalyst. Examples of other pretreatment techniques are dilute acid, alkaline and solvent pretreatment, the ammonia-freeze process, and wet oxidation.

In this study corn stover was used as the raw material. Corn stover with a dry matter content of 20% was impregnated with 2% SO<sub>2</sub> (w/w, based on the water content in the material) and then steam pretreated at 170, 180, 190, 200, and 210°C for 2, 5, and 10 min. The pretreatment was evaluated by enzymatic hydrolysis of the solid material and by fermentation of the liquid fraction.

## Materials and Methods

The experimental procedure used is shown schematically in Fig. 1. Samples were withdrawn at various stages in the experiment (SA = Samples for analysis) and later analyzed.

The dried corn stover was first presteamed to increase the water content. It was then impregnated with SO<sub>2</sub> and steam pretreated. The resulting slurry was separated into a solid and a liquid fraction. The liquid fraction was analyzed with respect to monomeric sugars, oligomeric sugars, and inhibitors such as acetic acid, furfural, and 5-hydroxymethyl-2-furfural (HMF). A fermentation test of the liquid fraction was also carried out to investigate the effect of inhibitors on the yeast. The solid fraction was washed and enzymatically hydrolyzed to determine the effect of the pretreatment.

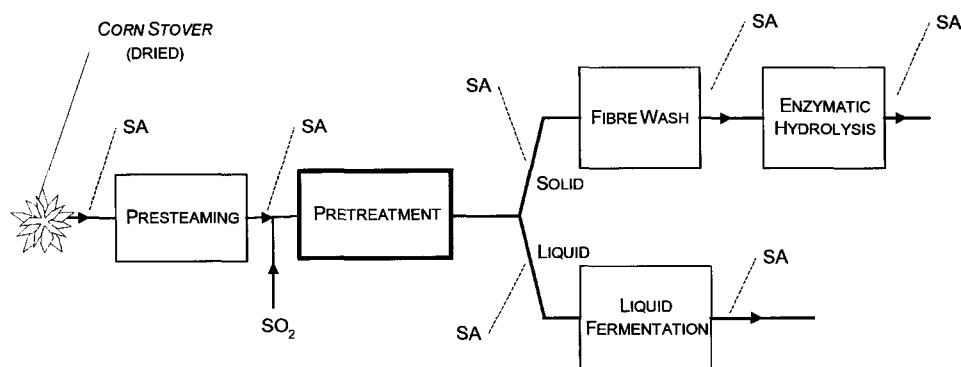


Fig. 1. Experimental procedure.

### Raw Material

The corn stover used in this investigation was grown in Hungary. It was collected in the autumn of 2002, after the harvest of the mature crop. After collection, the corn stover was chopped, air-dried, and then stored at room temperature. The sugar content and the lignin content of the raw material were determined using the analytical procedures developed at the National Renewable Energy Laboratory (NREL) (6–10). The concentration of monosaccharides in the clear solution was analyzed using high-performance liquid chromatography (HPLC), and the concentration of acid-soluble (AS) lignin was analyzed spectrophotometrically. The amount of acid-insoluble (AI) lignin was determined when the remaining solution was filtered through glass-fiber filters and washed with hot distilled water. The remaining solid fraction was dried at 105°C overnight before weighing.

### Steam Pretreatment

Prior to pretreatment the material was milled and sieved and the fraction between 2 and 10 mm was used. Because the raw material used was very dry (95% DM, owing to the dry storage) it had to be remoistened. This was done by presteaming with saturated steam at 100°C for 30 min, after which the material was immersed in cold water. The dried and rewetted material probably differ slightly from fresh, moist material because some collapsed pores may not regain their original shape and the pore size has an impact on the subsequent enzymatic hydrolysis (11).

The moistened corn stover was impregnated with SO<sub>2</sub> in plastic bags and the uptake was measured by weighing the material before and after impregnation. Adding 3% SO<sub>2</sub> (w/w, based on the water content of the corn stover) resulted in an actual uptake of around 2% SO<sub>2</sub>. The impregnated raw material was then steam pretreated in a 2.4-L reactor for 2, 5, or 10 min after which the material was collected for subsequent analysis. The

time to reach the desired temperature was in all cases less than 30 s. A total of 15 different pretreatment combinations were used: 170, 180, 190, 200, and 210°C for 2, 5, and 10 min.

### *Enzymatic Hydrolysis*

The solid fraction of the pretreated material was washed with warm tap water and enzymatically hydrolyzed to determine the potential sugar yield. A commercial cellulase mixture, Celluclast 1.5 L (65 FPU/g mixture and 17  $\beta$ -glucosidase IU/g mixture), was used, supplemented with the  $\beta$ -glucosidase preparation Novozyme 188 (376  $\beta$ -glucosidase IU/g preparation), both kindly donated by Novozymes A/S (Bagsværd, Denmark). Hydrolysis was performed at 40°C for 96 h using a dry matter concentration of 2% to avoid end product inhibition of the enzymes.

Ten grams dry matter (DM), 2.32 g Celluclast 1.5 L, and 0.52 g Novozym 188 were added to a 0.1 mol/L NaAc buffer (pH 4.8) to a total weight of 500 g, after which the flasks were placed in a water bath. Samples were taken after 0, 2, 4, 6, 8, 24, 48, 72, and 96 h and analyzed for monosaccharides.

### *Fermentation*

The liquid fraction following pretreatment was fermented to investigate the fermentability and the extent of inhibition. The pH was adjusted to 5.5 with 20% Ca(OH)<sub>2</sub> solution and glucose was added to the liquids to adjust the concentration of glucose to 50 g/L. A reference solution containing 50 g/L glucose was also fermented, and the fermentation experiments were all performed in duplicate.

Compressed baker's yeast, *Saccharomyces cerevisiae* (Jästbolaget AB, Rotebro, Sweden), was used at an initial concentration of 5 g DM/L. This yeast ferments hexoses, i.e., glucose and mannose, but not pentoses. Glass flasks of 25 mL with a working volume of 20 mL were used to ferment a mixture consisting of 18.5 mL filtrate and 1 mL inoculum (containing 100 g dry matter yeast/L). A volume of 0.5 mL nutrients was added to give a final concentration of 0.5 g/L (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.025 g/L MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1 mol/L NaH<sub>2</sub>PO<sub>4</sub>, and 1 g/L yeast extract. The flasks were sealed with rubber stoppers through which hypodermic needles were inserted for the removal of the CO<sub>2</sub> produced and to take samples. The flasks were incubated at 30°C for 24 h and samples were withdrawn after 0, 2, 4, 6, 8, and 24 h and analysed for ethanol, glucose, and acetic acid and sugar degradation products such as furfural and HMF.

### *Analysis*

The amounts of monosaccharides and inhibitors were determined by HPLC. Glucose, arabinose, galactose, and xylose were separated using an Aminex HPX-87-Pb column (Bio-Rad, Hercules, CA, USA) at 85°C and a

Table 1  
Composition of Corn Stover Expressed as % of Dry Matter

Glucan	Xylan	Arabinan	Galactan	AI lignin <sup>a</sup>	AS lignin
36.8 ± 1.2	22.2 ± 0.8	5.5 ± 0.9	2.9 ± 1.0	19.8 ± 0.5	3.3 ± 0.2

<sup>a</sup>Lignin ash included.

Table 2  
Reported Compositions of Corn Stover Expressed as % of Dry Matter

References	Glucan	Xylan	Arabinan	Galactan	AI lignin	AS lignin
Kaar et al. (20)	39.0	20.1	2.0	—	21.5	
Esteghlalian et al. (21)	36.0	19.8	2.8	1.3	17.8	1.9
Torget et al. (14)	40.9	21.5	1.8	1.0	16.7	
Schell et al. (22)	38.6	20.4	—	—	—	—
Kalman et al. (13), batch 1	42.5	16.1	3.5	—	20.1	
Kalman et al. (13), batch 2	43.8	20.3	2.2	—	16.8	

flow rate of 0.5 mL/min with water as eluent. Glucose, arabinose, lactic acid, glycerol, acetic acid, ethanol, HMF, and furfural were separated on an Aminex HPX-87-H column at 65°C using 5 mmol/L H<sub>2</sub>SO<sub>4</sub> as eluent at a flow rate of 0.5 mL/min. The samples from the liquid fraction after pretreatment were neutralized using CaCO<sub>3</sub> and Ba(OH)<sub>2</sub> and diluted three times. Ba(OH)<sub>2</sub> was used to precipitate sulfur, and CaCO<sub>3</sub> was used for the final pH adjustment. All samples were filtered through a 0.20-μm filter before analysis.

To measure the total amount of sugars (monomers and oligomers) in the liquid fraction after pretreatment, the samples were hydrolyzed with 4% H<sub>2</sub>SO<sub>4</sub> at 121°C for 1 h and then neutralized using Ca(CO)<sub>3</sub>. They were then diluted six times and analyzed with HPLC using an Aminex HPX-87-Pb column as described above.

The amount of acid-soluble lignin was determined using an absorption spectrophotometer at a wavelength of 205 nm with a 4% H<sub>2</sub>SO<sub>4</sub> solution as a reference.

## Results and Discussion

### Raw Material

Results from the analysis of the raw material composition are presented in Table 1. Fifty-nine percent of the material consisted of glucan and xylan. Thomas (12) who analyzed over 700 corn stover samples in the US, found that the range of carbohydrate content in corn stover is large, and depends on both environmental and genetic factors. They also found that a corn

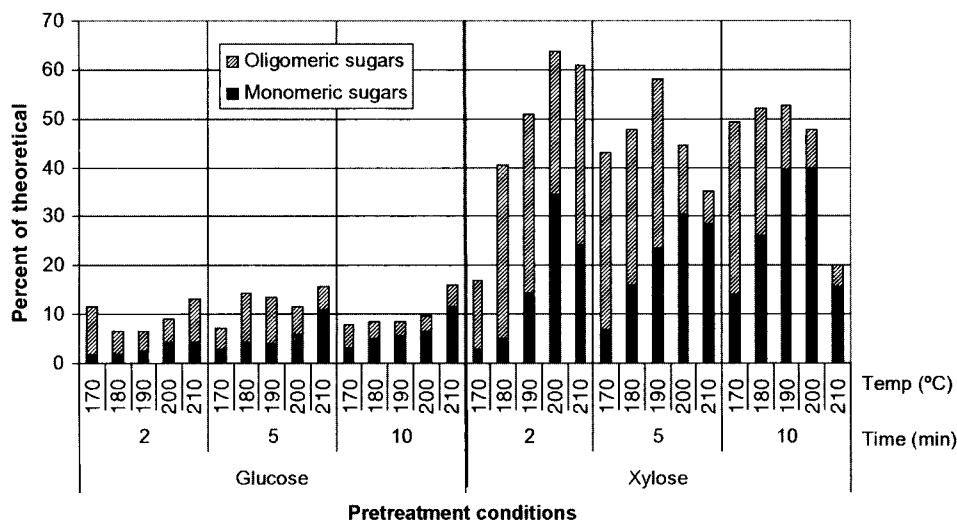


Fig. 2. Xylose and glucose yields in the pretreatment liquid, expressed as % of theoretical yield.

stover sample from one family that had a high content of glucan did not necessarily have a high content of xylan, because the weight fractions of the five major sugar components in corn stover were fairly independent of each other. Table 2 presents six reported composition analyses. The corn stover used in the present investigation had an arabinan content that was significantly higher, a xylan content that was slightly higher, and a glucan content that was slightly lower, than those presented in Table 2.

### Steam Pretreatment

The water-insoluble dry matter content in the slurry after pretreatment varied between 12%, for the mildest conditions (2 min, 170°C), and 5% for the harshest conditions (10 min, 210°C). The amount of liquid in the slurry varied between 1.1 L for the mildest conditions and 1.4 L for the harshest (results not shown). This, in combination with the fact that the amount of water-insoluble dry matter varies between 149 g for the mildest conditions and 79 g for the harshest conditions, shows that at harsher conditions, more of the fibers are degraded, resulting in more sugar being liberated, and probably also in more degradation products. Figure 2 shows the yields of xylan and glucan hydrolyzed during the pretreatment, present in the liquid phase as monomeric and oligomeric sugars.

More xylose than glucose was found in the liquid because hemicellulose is more easily hydrolyzed than cellulose. Sixty-four percent of the theoretical amount of xylan was hydrolyzed when the material was pretreated at 200°C for 2 min, and almost as much, 61 and 58% of the xylan, was hydrolyzed at 210°C for 2 min and 190°C for 5 min, respectively. The maximum hydrolyzed fraction of glucan was 16%, obtained with pretreat-

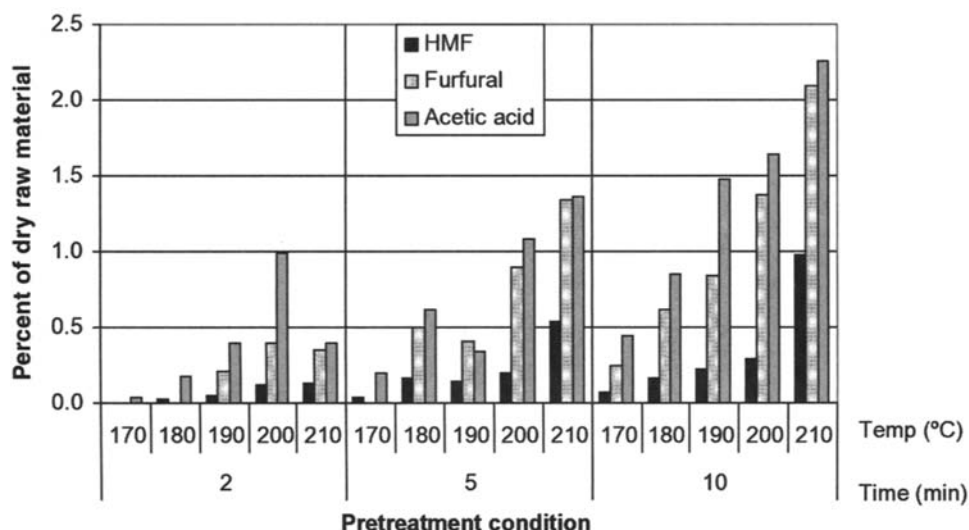


Fig. 3. Formation of inhibitors during pretreatment.

ment at 210°C for 10 min and at 200°C for 5 min. Kalman et al. (13) reported complete solubilization of pentoses and 11% solubilization of glucose in samples that were steam pretreated with dilute sulfuric acid at 120°C for 90 min.

When glucose and xylose are further degraded in an acidic environment at high temperature and pressure, furfural and HMF are formed, which causes the concentration of xylose in the liquid to fall with harsher pretreatment conditions. Figure 2 shows that when pretreatment was carried out for 2 min the amount of xylose started to decrease at 200°C due to degradation and when using 10 min pretreatment, the amount of xylose started to decrease already at 180–190°C and at 210°C it was significantly reduced.

The amount of inhibitors formed in the pretreatment step is shown in Fig. 3 as a percentage of the initial dry raw material. Torget et al. (14) found a 1.9% (on a dry basis) content of acetyl groups in corn stover. Hydrolyzing oligomeric xylan to monomeric xylose thus results in the liberation of the acetyl groups forming acetic acid. Harsher pretreatment conditions will evidently result in more acetic acid in the liquid until no oligomeric xylan, and thus acetyl groups, remain in the material. Figure 2 shows that there is still oligomeric xylan in the liquid at all conditions, which explains the increase in acetic acid concentration throughout the study, as can be seen in Fig. 3.

The amount of acetic acid produced when corn stover was pretreated at 210°C for 10 min corresponds to a concentration in the liquid fraction after pretreatment of 2.9 g/L. The degradation into furfural and HMF at the same conditions (210°C, 10 min) corresponds to concentrations of 2.5 and 1.2 g/L, respectively. These concentrations are also the highest concentrations (g/L) found in the liquid fraction after pretreatment.

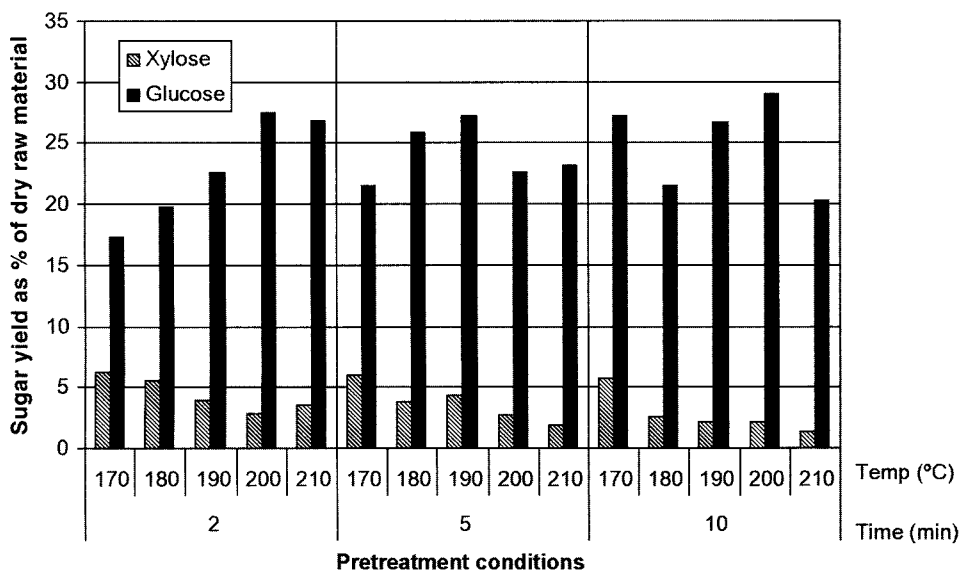


Fig. 4. Conversion of glucan and xylan in enzymatic hydrolysis.

### Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out to evaluate the pretreatment conditions. Harsher pretreatment conditions resulted in a lower amount of sugars in the solid fraction for the enzymes to hydrolyze. Figure 4 shows the percentage of the dry raw material that was converted to monomeric sugars during enzymatic hydrolysis.

The amount of xylose liberated during the enzymatic hydrolysis decreased with increasing harshness of the pretreatment conditions. Thus severe pretreatment left very little xylose in the solid material. Enzymatic hydrolysis of material pretreated at 210°C for 2 min resulted in a xylose yield of 97%. Although high, this yield corresponds to only 3.5% of the original dry raw material, see Fig. 4.

Figure 6 shows that the glucose yield varied between 58%, for pretreatment conditions 170°C for 2 min, to between 80 and 90%, for pretreatment at 200°C for various times. It is difficult to measure the dry matter content in the material after pretreatment at low temperature as there is a large amount of fibrous material present, which results in uneven liquid distribution.

Varga et al. (15) obtained a glucose yield from the enzymatic hydrolysis step of up to 95.7% for optimized two-step pretreatment of corn stover using soaking in 1% NaOH followed by pretreatment with 1% H<sub>2</sub>SO<sub>4</sub> at 120°C for 1 h with 10% dry matter. Using one-step pretreatment at 120°C for 1 h with NaOH as catalyst, the highest yield reported was 79.4%. With HCl as a catalyst, the highest conversion was 32.4%, and with H<sub>2</sub>SO<sub>4</sub>, it was 46.2% (all pretreatments carried out at 120°C for 1 h).



In the present study the highest enzymatic hydrolysis conversion occurred when the material was pretreated at 210°C for 2 min. The yield of glucose was 90% and that of xylose 97%. The material pretreated for 2 min shows a steady increase in glucose and xylose yield to the maximum at 210°C, see Fig. 4. Following short pretreatment, there are still significant amounts of both glucose and xylose remaining in the solid material, which makes the results more reliable. After longer pretreatment and at higher temperatures, the amount of xylose in the solid material is so low that accurate analysis is difficult.

### *Fermentation*

Fermentability tests were carried out to determine the inhibiting effect of the liquid fractions resulting from pretreatment. In this study, a 100% yield of ethanol (0.51 g ethanol/g glucose) was found for all liquids except that resulting from pretreatment at the most severe conditions, 210°C, 10 min, where no ethanol production was detected. This agrees well with results obtained by Larsson et al.(16) who showed that furfural and HMF in moderate concentrations did not affect the ethanol yield, only the productivity. The ethanol yield in the reference solutions varied between 80 and 95%.

Furfural inhibits glucose fermentation but it can be metabolized by yeast to furfuryl alcohol which shows very small inhibitory effects (16). When furfural is metabolized the fermentation rate of glucose is very low resulting in a delay in fermentation until all the furfural has been metabolized. The highest concentration of furfural in the liquid fraction before fermentation was 2.5 g/L found after pretreatment at 210°C for 10 min. This amount of furfural was not metabolized at all, even after 24 h. A concentration of 1.7 g/L furfural (200°C, 10 min) was metabolized in 8 hours and 1.5 g/L furfural (210°C, 5 min) was completely metabolized in 2 hours. Larsson et al. (1999) (16) found that a solution with only 4.6 g/L (48 mmol/L) furfural decreased the productivity but was completely metabolized in 2 h. This reduction is possible with furfural concentrations up to 10 g/L if no other inhibiting compounds are present(17). Additive effects are probably the reason for the absence of yeast activity in the liquid from the pretreatment at 210°C for 10 min.

HMF is not metabolised to the same extent as furfural, as was also observed by Larsson et al. (16). The highest concentration of HMF that was completely metabolised in 24 h in the present study was found to be 0.4 g/L (200°C, 10 min). In liquid from pretreatment at 210°C for 10 min for the concentration of HMF was 1.2 g/L and it was not significantly reduced during fermentation.

Other inhibiting products such as acetic acid and degradation products such as formic acid and levulinic acid, formed when HMF and furfural are further degraded, have been shown to increase the ethanol yield when present at concentrations up to 100 mmol/L while higher concentrations

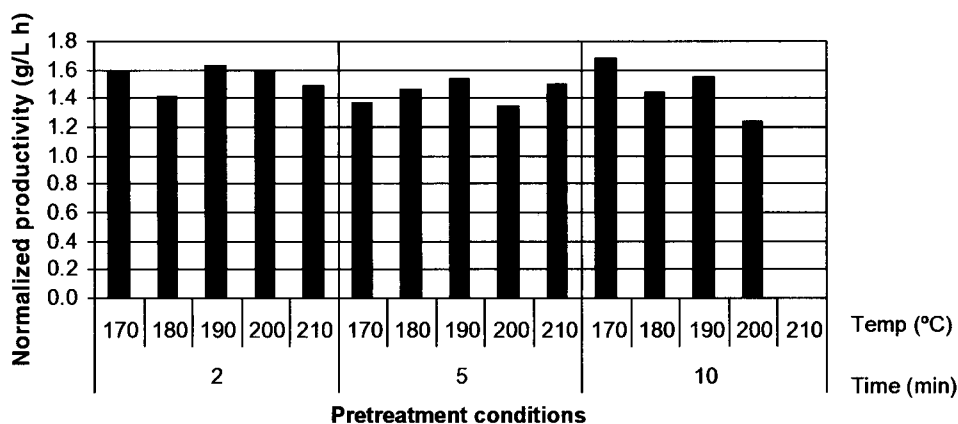


Fig. 5. Normalized ethanol productivity.

decreased the ethanol yield (16). Low concentrations of undissociated acids tend to increase the ethanol productivity since the yeast cells become stressed, while high concentrations are fatal to the yeast (18).

Figure 5 shows the average productivity after two hours normalized with respect to the productivity in the reference solution. It varied between 1.2 and 1.7, except for the liquid from the most severe pretreatment conditions (210°C, 10 min) where no productivity was observed.

### Overall Yields

Figure 6 shows the overall yields of xylose and glucose, calculated from the monosaccharides hydrolyzed in the enzymatic hydrolyze ( $\text{Sugar}_{\text{EH}}$ ), the monosaccharides hydrolyzed in the pretreatment ( $\text{Sugar}_{\text{(pre)}}$ ), and the total amount of sugar in the raw material ( $\text{Sugar}_{\text{RM}}$ ) according to:

$$\text{Overall yield} = \frac{[\text{Sugar}_{\text{EH}}] + [\text{Sugar}_{\text{(pre)}}]}{[\text{Sugar}_{\text{RH}}]} \quad (1)$$

The overall yield of glucan was rather high for a broad range of pretreatment conditions (see fig. 6).

The highest yield, 89%, was obtained when corn stover was pretreated at 200°C for 10 min. These yields are high compared with, for example, those found by Varga et al. (15) who evaluated pretreatment using different liquid chemicals ( $\text{H}_2\text{SO}_4$ , HCl, and NaOH) and concluded that treatment with 0.5% NaOH at 120°C for 90 min resulted in an overall sugar yield (after hydrolysis) of 83.3%. Two-step pretreatment using 1%  $\text{H}_2\text{SO}_4$  after soaking for 1 d in 1% NaOH gave an overall sugar yield of 89.7%. In the present study, it was only at very mild pretreatment conditions (170°C for 2 min; 170°C for 5 min, or 180°C for 5 min) that the yields differed considerably from the maximum yield.

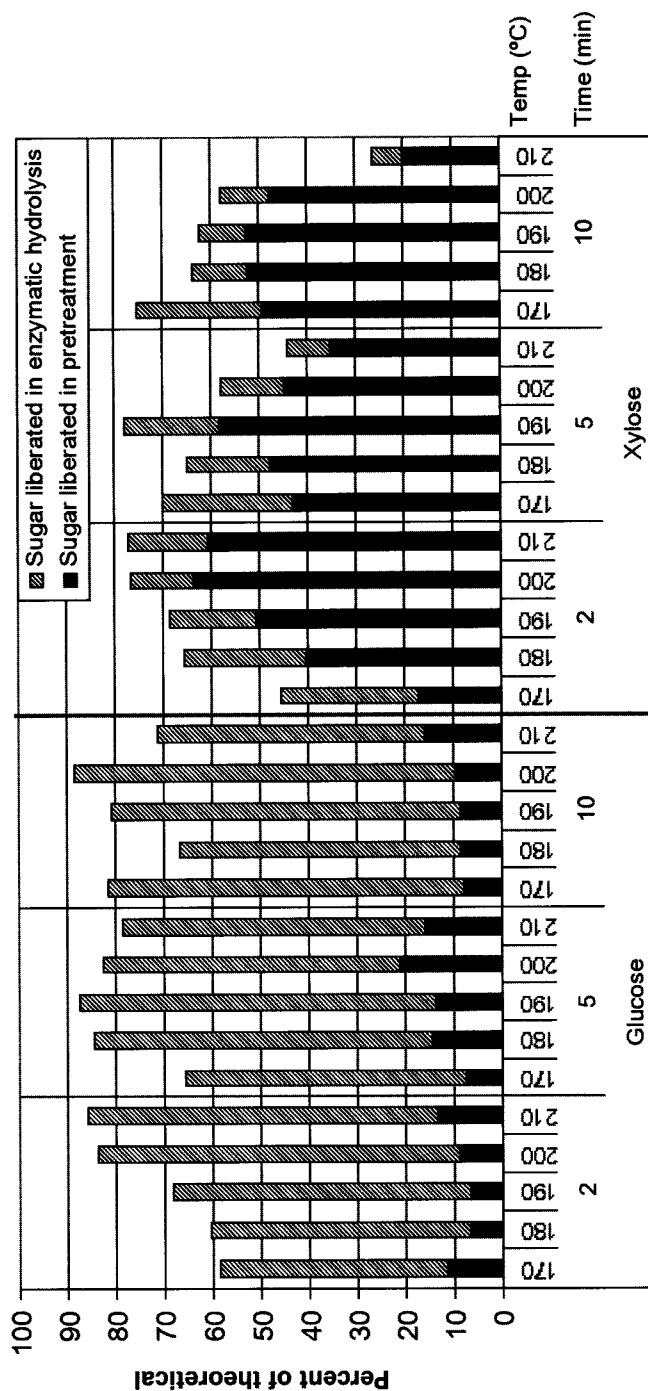


Fig. 6. Overall yields of glucose and xylose.

The glucose yield achieved when the material was pretreated at 170°C for 10 min was unexpectedly high. This may be due to the problem of measuring a representative dry matter content of material pretreated at low temperatures since the material is still very fibrous and the liquid distribution uneven.

The overall xylan yield was 78% when corn stover was pretreated at 190°C for 5 min; 77% at 210°C for 2 min; 77% at 200°C for 2 min, and 75% when pretreated at 170°C for 10 min (see Fig. 6). The xylose yield achieved when the material was pretreated at 170°C for 5 min was higher than expected. These yields were all lower than those obtained for glucan, but still rather high for one-step pretreatment. Kalman et al. (13) reported a 100% overall conversion of pentoses when using dilute sulfuric acid pretreatment at long residence times (90–120 min) and low temperature (120°C) but they achieved only 54.1% overall glucose yield. However, Tucker et al. (19) found xylose yields above 90% with dilute sulfuric acid steam pretreatment at 190°C.

In the present study, it was only at pretreatment conditions that were very mild (170°C for 2 min) or very severe (200°C for 5 min, 200°C for 10 min, 210°C for 5 min, or 210°C for 10 min) that the yields deviated significantly from the maximum yield. The highest total overall yields of glucose and xylose in the present study were found to be 84% (87% glucose yield and 78% xylose yield), obtained when the material was pretreated at 190°C for 5 min.

## Conclusions

Corn stover, with its high sugar content, is a good raw material for ethanol production provided that an organism capable of fermenting both hexoses and pentoses is available for the fermentation of sugars to ethanol. This study showed that a high overall yield of both glucose (87%) and xylose (78%) after enzymatic hydrolysis could be achieved with one-step steam explosion pretreatment using SO<sub>2</sub> as a catalyst.

High temperature and short time in the pretreatment step has been shown to favor a high glucose yield, and low temperature and long time to favor a high xylose yield. The highest overall yield of glucose and xylose, 84%, was obtained for pretreatment at 190°C for 5 min. In this case 1000 kg dry corn stover would, after pretreatment and hydrolysis, result in 556 kg sugar (glucose and xylose). If a 90% yield in the fermentation is assumed, then 322 L (255 kg) ethanol could be produced.

## Acknowledgment

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